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(21) International Application Number: PCT/GB00/00503 (22) International Filing Date: 15 February 2000 (15.02.00) (30) Priority Data: 9903403.5 16 February 1999 (16.02.99) GB (71) Applicant (for all designated States except US): ANGIOGENE PHARMACEUTICALS LTD. [GB/GB]; 14 Plowden Park, Aston Rowant, Watlington, Oxfordshire OX9 5SW (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): DAVIS, Peter, David [GB/GB]; 10 Aston Park, Aston Rowant, Watlington OX9 5SW (GB). (74) Agents: BAILLIE, Iain, C. et al.; Langner Parry, 52-54 High Holborn, London WC1V 6RR (GB).		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: SUBSTITUTED STILBENE COMPOUNDS WITH VASCULAR DAMAGING ACTIVITY (57) Abstract Vascular damaging agents for use in treating diseases involving angiogenesis are provided which are compounds of formula: A-X-B, wherein A is a substituted cis-stilbene moiety, X a linker bond, atom or group and B is a moiety derived from an inhibitor of the formation or action of nitric oxide in mammalian systems specifically an inhibitor of nitric oxide synthase, and hydrates, pharmaceutically acceptable salts and prodrugs thereof. There are also provided compositions containing such compounds.		

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SUBSTITUTED STILBENE COMPOUNDS WITH VASCULAR DAMAGING ACTIVITY

This invention relates to vascular damaging agents and particularly to a series of novel
5 stilbene compounds.

Formation of new vasculature by angiogenesis is a key pathological feature of several diseases (J Folkman, New England Journal of Medicine 333, 1757-1763, 1995). For example, for a solid tumour to grow it must develop its own blood supply upon which
10 it depends critically for the provision of oxygen and nutrients; if this blood supply is mechanically shut off the tumour undergoes necrotic death. Neovascularisation is also a clinical feature of skin lesions in psoriasis, of the invasive pannus in the joints of rheumatoid arthritis patients and of atherosclerotic plaques. Retinal neovascularisation is pathological in macular degeneration and in diabetic retinopathy. In all these diseases
15 reversal of neovascularisation by damaging the newly-formed vascular endothelium is expected to have a beneficial therapeutic effect.

Combretastatin A4 phosphate is an agent known to have vascular damaging activity in animal models of solid tumours (Dark et al, Cancer Research 57, 1829-1834, 1997).
20 However some tumours are resistant to this agent and doses approaching the maximum tolerated dose are necessary to produce significant vascular damage in these tumours.

One characteristic of tumours resistant to combretastatin A4 phosphate is their ability
25 to produce large amounts of nitric oxide. The role of nitric oxide in tumour growth is unclear and there have been reports of both tumour-stimulating and tumour-inhibiting effects (Chinje and Stratford, Essays Biochem. 32, 61-72, 1997).

The present invention concerns novel combretastatin derivatives, methods for their
30 preparation, pharmaceutical compositions containing them and their use as vascular damaging agents for the treatment of diseases involving active angiogenesis. These derivatives are more active than combretastatin A4 phosphate, particularly on tumours

that are resistant to the known vascular damaging agents. In solid tumours vascular damaging agents exert their anti-tumour effect largely by inducing necrosis in the tumour, through starvation of the tumour's blood supply. Compounds of the invention show improved activity in the induction of necrosis in solid tumours. Though not
5 limiting on the invention it is believed that the ability of compounds of the invention to reduce the production of nitric oxide during vascular damage by inhibition of one or more of the enzymes that produce nitric oxide (the nitric oxide synthases), is one way in which the compounds achieve increased activity.

10 Thus in one embodiment of the invention there is provided a compound of formula IA



IA

15

Wherein

A is a substituted *cis*-stilbene moiety

X is a linker bond, atom or group

20 B is a moiety derived from an inhibitor of the formation or action of nitric oxide in mammalian systems

and the hydrates, pharmaceutically acceptable salts and prodrugs thereof.

25 In a more specific embodiment of the invention there is provided a vascular damaging agent which is a compound of formula I



30

I

Wherein

A is a substituted *cis*-stilbene moiety

X is a linker bond, atom or group

B is a moiety derived from an inhibitor of nitric oxide synthase

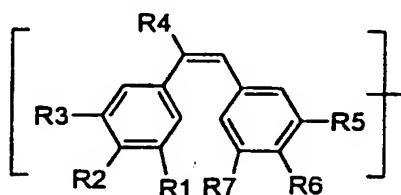
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and the hydrates, pharmaceutically acceptable salts and prodrugs thereof.

The linker X can be attached to any available atom of the stilbene moiety A and to any available atom of nitric oxide synthase inhibitor B as appropriate.

10

The stilbene moiety A can be for example a group of formula II



II

Wherein

15

R1, R2 and R3 are each independently H, optionally substituted alkoxy, optionally substituted alkyl or halogen

R4 is hydrogen or cyano

20 R5, R6 and R7 are each independently H, hydroxy, optionally substituted alkoxy, optionally substituted alkyl, halogen, amino, alkylamino, dialkylamino, cyano, nitro, carboxyl, alkanoyl, alkoxycarbonyl, alkoxycarbonyloxy, alkoxycarbonylamino, aminocarbonylamino, alkylaminocarbonylamino, dialkylaminocarbonylamino, alkylcarbonylamino, alkylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl, alkylsulphonylamino, aminosulphonylamino, 25 alkylaminosulphonylamino, dialkylaminosulphonylamino, mercapto, alkylsulphanyl or alkylsulphinyl,

with the proviso that at least two of R1, R2 and R3 must be optionally substituted alkoxy.

Stilbene moiety A can be attached to linker group X by any available valency.

5

Linker group X can be for example a bond, an optionally substituted methylene chain, or $-(CH_2)_m-Y-(CH_2)_n-$ wherein Y is selected from -O-, -S-, -S(O)-, -SO₂-, -NH-, -Nalkyl-, -CO-, -OC(O)-, -NHC(O)-, -N(alkyl)C(O)-, -NHC(O)NH-, -NalkylC(O)NH-, -NalkylC(O)Nalkyl-, -NHSO₂-, -NalkylSO₂-, -NHSO₂NH-, -NalkylSO₂NH-, -

10 NalkylSO₂Nalkyl- and -OC(O)O-, m is 0-3 and n is 0-3. Where the group Y is not symmetrical it can be oriented in either direction such that either end can be attached to the group A.

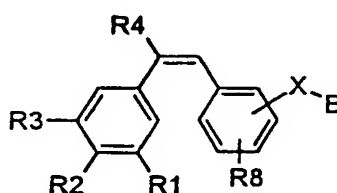
The nitric oxide synthase inhibitor moiety B can be a group derived from an inhibitor
15 of nitric oxide synthase. Such inhibitors include, for example a group derived from an amino acid inhibitor of nitric oxide synthesis for example a group -C(O)CH(NH₂)-(CH₂)_p-NHC(NH)Z wherein p is 1-5 and Z is alkyl, alkylamino, dialkylamino, nitroamino, hydrazino or alkylthio, or for example a group -NHCH(CO₂R₁₀)-(CH₂)_p-NHC(NH)Z where p and Z are as hereinbefore described and R₁₀ is hydrogen or alkyl.

20 A further example of a nitric oxide synthase inhibitor moiety B is a group derived from thiocitrulline for example a group -C(O)CH(NH₂)-(CH₂)_p-NHC(S)NH₂ or a group -NHCH(CO₂R₁₀)-(CH₂)_p-NHC(S)NH₂. A further example of a nitric oxide synthase inhibitor moiety B is a group derived from an S-alkylisothioureia for example -(CH₂)_p-SC(NH)NH₂. A further example of a nitric oxide synthase inhibitor moiety B is a
25 group derived from a 2-aminopyridine for example a 4-methyl-2-pyridinylamino group.

As used herein the term "alkyl", alone or in combinations, means a straight or branched-chain alkyl group containing from one to seven, preferably a maximum of four, carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, t-butyl
30 and pentyl. Examples of alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy and t-butoxy. The term "halogen" means fluorine, chlorine, bromine or iodine.

Optionally substituted alkoxy groups, optionally substituted alkyl groups and optionally substituted methylene chains may bear one or more substituents independently selected from halogen, hydroxy, amino, alkylamino, dialkylamino, carboxyl, mercapto, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylcarbonylamino, alkylcarbonyl(alkyl)amino, sulphate and phosphate.

One group of preferred compounds are those of formula III



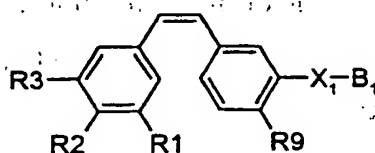
III

Wherein

R1, R2, R3, R4, X and B are as hereinbefore described
 R8 is alkyl, amino, hydroxy, alkoxy or halogen

A further preferred group of compounds are those of formula III wherein R1, R2, R3, R4, are as hereinbefore described, R8 is alkyl, amino, hydroxy, alkoxy or halogen, X is -O- or -NH- and B is a group $-C(O)CH(NH_2)-(CH_2)_p-NHC(NH)Z$ wherein p is 1-5 and Z is alkyl, alkylamino, dialkylamino, nitroamino, hydrazino or alkylthio or a group $-NHCH(CO_2R_{10})-(CH_2)_p-NHC(NH)Z$ where p, Z and R10 are as hereinbefore described.

A still further preferred subset includes compounds of formula IV



IV

Wherein

R1, R2 and R3 are as hereinbefore described

R9 is alkyl, alkoxy or halogen

X₁ is O or NH

- 5 B₁ is a group -C(O)CH(NH₂)-(CH₂)_p-NHC(NH)Z wherein p is 1-5 and Z is alkyl, alkylamino, dialkylamino, nitroamino, hydrazino or alkylthio.

Particularly preferred compounds include:

- (Z)-1-(4-Methoxy-3-N^G-nitroarginyloxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene
- 10 (Z)-N-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)ethenyl]phenoxy carbonyl]N^G-nitroarginine methyl ester
- (Z)-N-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)ethenyl]phenoxy carbonyl]N^G-nitroarginine
- (Z)-N-[2-methyl-5-[2-(3,4,5-trimethoxyphenyl)ethenyl]phenoxy carbonyl]N^G-
- 15 nitroarginine methyl ester

- For the avoidance of doubt it is to be understood that where in this specification a group is qualified by "hereinbefore defined" or "defined hereinbefore", or "hereinafter defined" or "defined hereinafter", the said group encompasses the first occurring and
- 20 broadest definition as well as each and all of the preferred definitions for that group.

- Where one or more functional groups in compounds of formula I are sufficiently basic or acidic the formation of salts is possible. Suitable salts include pharmaceutically acceptable salts for example acid addition salts including hydrochlorides,
- 25 hydrobromides, phosphates, sulphates, hydrogen sulphates, alkylsulphonates, arylsulphonates, acetates, benzoates, citrates, maleates, fumarates, succinates, lactates and tartrates, salts derived from inorganic bases including alkali metal salts such as sodium or potassium salts, alkaline earth metal salts such as magnesium or calcium salts, and salts derived from organic amines such as morpholine, piperidine or
- 30 dimethylamine salts.

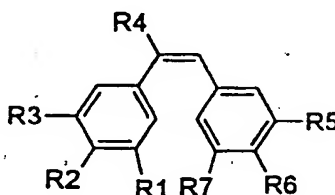
Compounds of formula I or IA or a salt thereof may exhibit tautomerism and the formulae drawings within this specification represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form that has vascular damaging activity and is not to be limited merely to
5 any one tautomeric form utilised within the formulae drawings.

Those skilled in the art will recognise that compounds of formula I or IA may exist as stereoisomers and accordingly the present invention includes all such isomers and mixtures thereof which have vascular damaging activity. Where the group derived
10 from a nitric oxide synthase inhibitor is derived from an amino acid inhibitor of nitric oxide synthase the L-configuration of the amino acid is preferred.

Compounds of the invention can be prepared by any process known to a person skilled in the art. Compounds of formulae IA, I, III and IV can be prepared by a number of
15 processes as generally described hereinbelow and more specifically in the Examples hereinafter. In the general preparations described below it may be necessary to employ protecting groups which are then removed during the final stages of the synthesis. The appropriate use of such protecting groups and processes for their removal will be readily apparent to those skilled in the art. In the following process description, the
20 symbols R1, R2, R3, R4, R5, R6, R7, X and B when used in the formulae depicted are to be understood to represent those groups described above in relation to formula I unless otherwise indicated

Thus according to a further aspect of the invention compounds of the invention may be
25 prepared by attachment of a nitric oxide synthase inhibitor to a stilbene of formula V using alkylation, acylation, sulphonylation or coupling reactions. Alternatively stilbenes of formula V may be coupled to a difunctional compound (which provides the linker group -X-) and further coupled to the nitric oxide inhibitor via the remaining
30 functionality on the linker group as appropriate. Stilbenes of formula V are either known or can be prepared using methods analagous to those used in the preparation of the known stilbenes which will be apparent to those skilled in the art.

In one general example compounds of formulae I can be prepared from a stilbene of formula V containing a free OH or NH by acylation with a nitric oxide synthase inhibitor containing a carboxylic acid for example using a coupling agent such as a carbodiimide, for example dicyclohexylcarbodiimide, or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and, optionally, a base such as an organic base for example triethylamine and, optionally, a catalyst such as 4-dimethylaminopyridine in a solvent such as an aprotic solvent for example dimethylformamide or in a chlorinated solvent for example chloroform or dichloromethane at a temperature in the range from about -30°C to about 60°C, conveniently at or near room temperature.



In another general example a compound of formula V containing a free OH or NH group can be treated with 4-nitrophenylchloroformate in a solvent such as pyridine at a temperature of about -10°C to room temperature followed by treatment with a nitric oxide synthase inhibitor containing a free OH or NH group to give a compound of formula I containing a carbonate, carbamate or urea group.

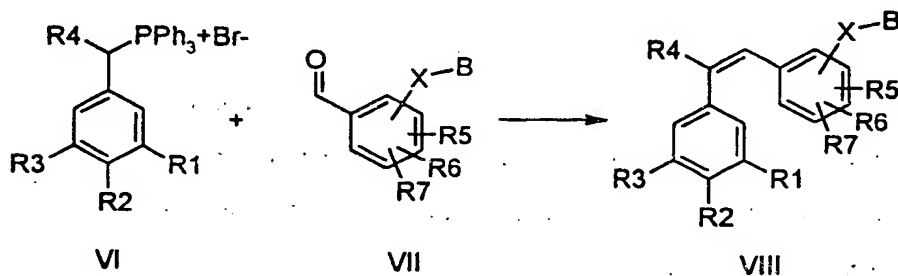
In another general example a compound of formula V containing a free NH group can be treated with a dicarboxylic acid monoester such as monomethyl succinate in the presence of a coupling agent such as a carbodiimide, for example dicyclohexylcarbodiimide, or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and, optionally, a base such as an organic base for example triethylamine in a solvent such as an aprotic solvent for example dimethylformamide or in a chlorinated solvent for example chloroform or dichloromethane at a temperature in the range from about -30°C to about 60°C, conveniently at or near room temperature. The resulting ester can be hydrolysed by treatment with aqueous acid or aqueous base under standard

conditions and the carboxylic acid so obtained treated with a nitric oxide inhibitor containing a free OH or NH group, using a coupling agent as described hereinbefore, to give compounds of the invention.

- 5 In another general example a compound of formula V containing a carboxylic acid group can be converted into a compound of formula I containing an amide or ester by treatment with a nitric oxide synthase inhibitor, containing an amino group or a hydroxyl group respectively, using a coupling agent as described hereinbefore.
- 10 In another general example a compound of formula V containing a monohaloalkyl group can be reacted with a nitric oxide synthase inhibitor containing a free OH, NH, or SH group in the presence of a base such as sodium carbonate or a metal hydride such as sodium hydride in a solvent such as dimethylformamide at a temperature of about 0°C to a temperature of about 100°C to give compounds of the invention.
- 15 In another general example a compound of formula V containing a carboxylic acid group can be treated with a monoprotected diamino, dihydroxy or aminohydroxy compound such as a monoprotected diaminoalkane, a monoprotected dihydroxyalkane or mono-protected aminohydroxyalkane, using a coupling agent as described
- 20 hereinbefore and the resulting amide or ester deprotected and reacted with a nitric oxide synthase inhibitor containing a carboxylic acid using a coupling agent as described hereinbefore.
- 25 In another general example a compound of formula V containing a free OH or NH group can be sulphonylated with a protected amino sulphonylchloride such as a protected aminoalkylsulphonylchloride or a protected hydroxy sulphonyl chloride such as a protected hydroxyalkylsulphonyl chloride, in the presence of a base, for example a tertiary amine base such as triethylamine, in for example a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -
- 30 30°C to 120°C, conveniently at or near ambient temperature and the resulting sulphonamide or sulphonate deprotected and reacted with a nitric oxide synthase inhibitor containing a carboxylic acid using a coupling agent as described hereinbefore.

In another general example a compound of formula V containing a free OH, SH or NH group can be alkylated with a difunctional alkylating agent such as a dihaloalkane in the presence of a base such as sodium carbonate or a metal hydride such as sodium hydride in a solvent such as dimethylformamide at a temperature of about 0°C to a temperature of about 100°C, and the resulting haloalkane further reacted under similar conditions with a nitric oxide synthase inhibitor containing a free OH, SH or NH group.

- Compounds of formula VII can also be prepared by Wittig olefin synthesis involving reaction of a phosphonium salt of formula VI with a strong base, for example an alkylolithium such as n-butyllithium or t-butyllithium or a metal hydride such as sodium hydride in a solvent such as an ether solvent for example diethyl ether or tetrahydrofuran or in a solvent such as a hydrocarbon solvent for example toluene at a temperature of between about -100°C to about 30°C followed by treatment with an aldehyde of formula VII.



- Compounds of formula I can also be prepared from other compounds of formula I by chemical modification. Examples of such chemical modifications that may be applied are standard alkylation, acylation, thioacylation, sulfonylation, aromatic halogenation and coupling reactions. These reactions may be used to add new substituents or to modify existing substituents. Alternatively, existing substituents in compounds of formula I may be modified by, for example, oxidation, reduction, elimination, hydrolysis or other cleavage reaction to yield other compounds of formula I.

Thus for example a compound of formula I containing an amino group may be acylated on the amino group by treatment with, for example, an acyl halide or anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example, a solvent such as a hydrocarbon solvent e.g. dichloromethane at a
5 temperature in the range for example -30°C to 120°C , conveniently at or near ambient temperature.

In another general example of an interconversion process an amino group in a compound of formula I may be sulphonylated by treatment with, for example, an alkyl
10 or aryl sulphonyl chloride or an alkyl or aryl sulphonic anhydride in the presence of a base, for example a tertiary amine base such as triethylamine, in for example a solvent such as a hydrocarbon solvent e.g. dichloromethane at a temperature in the range for example -30°C to 120°C , conveniently at or near ambient temperature.

15 In a further general example a compound of formula I containing an ester can be hydrolysed by treatment with an acid, for example sulphuric acid, in a solvent such as tetrahydrofuran in the presence of water at a temperature of about room temperature to the reflux temperature of the solvent, preferably at or around 60°C .

20 In a further general example a compound of formula I containing an amide can be hydrolysed by treatment with for example an acid such as hydrochloric acid in a solvent such as an alcohol, for example methanol at an elevated temperature conveniently at the reflux temperature.

25 In another general example an O-alkyl group may be cleaved to the corresponding alcohol (OH) by reaction with boron tribromide in a solvent such as a chlorinated solvent e.g. dichloromethane at a low temperature e.g. around -78°C .

In a further general example compounds of formula I may be alkylated by reaction with
30 a suitable alkylating agent such as an alkyl halide, an alkyl toluenesulphonate, an alkyl methanesulphonate or an alkyl triflate. The alkylation reaction can be carried out in

the presence of a base for example an inorganic base such as a carbonate e.g. caesium or potassium carbonate, a hydride such as sodium hydride or an alkoxide such as potassium t-butoxide in a suitable solvent such as an aprotic solvent e.g. dimethylformamide or an ether solvent such as tetrahydrofuran at a temperature of
5 around -10 to 80°C.

Preparation of a compound of formula I as a single enantiomer or, where appropriate, diastereomer may be effected by synthesis from an enantiomerically pure starting material or intermediate or by resolution of the final product in a conventional manner.

10 Acid addition salts of the compounds of formula I are prepared in a conventional manner by treating a solution or suspension of the free base I with about one equivalent of a pharmaceutically acceptable acid. Salts of compounds of formula I derived from inorganic or organic bases are prepared in a conventional manner by
15 treating a solution or suspension of the free acid I with about one equivalent of a pharmaceutically acceptable organic or inorganic base. Alternatively both acid addition salts and salts derived from bases may be prepared by treatment of the parent compound with the appropriate ion-exchange resin in a standard fashion. Conventional concentration and recrystallisation techniques are employed in isolating the salts.

20 Compounds according to the invention are able to destroy tumour vasculature and vasculature that has been newly formed while leaving unaffected normal, mature vasculature. The ability of the compounds to act in this way may be determined by the tests described in the Examples hereinafter.

25 The compounds according to the invention are thus of particular use in the prophylaxis and treatment of cancers involving solid tumours and in the prophylaxis and treatment of diseases where inappropriate angiogenesis occurs such as diabetic retinopathy, psoriasis, rheumatoid arthritis, atherosclerosis and macular degeneration.

30 The compounds of the invention may be administered as a sole therapy or in combination with other treatments. For the treatment of solid tumours compounds of

the invention may be administered in combination with radiotherapy or in combination with other anti-tumour substances for example those selected from mitotic inhibitors, for example vinblastine, paclitaxel and docetaxel; alkylating agents, for example cisplatin, carboplatin and cyclophosphamide; antimetabolites, for example 5-fluorouracil, cytosine arabinoside and hydroxyurea; intercalating agents for example adriamycin and bleomycin; enzymes, for example asparaginase; topoisomerase inhibitors for example etoposide, topotecan and irinotecan; thymidylate synthase inhibitors for example raltitrexed; biological response modifiers for example interferon; antibodies for example edrecolomab; and anti-hormones for example tamoxifen. Such combination treatment may involve simultaneous or sequential application of the individual components of the treatment.

For the prophylaxis and treatment of disease the compounds according to the invention may be administered as pharmaceutical compositions selected with regard to the intended route of administration and standard pharmaceutical practice. Such pharmaceutical compositions may take a form suitable for oral, buccal, nasal, topical, rectal or parenteral administration and may be prepared in a conventional manner using conventional excipients. For example for oral administration the pharmaceutical compositions may take the form of tablets or capsules. For nasal administration or administration by inhalation the compounds may be conveniently delivered as a powder or in solution. Topical administration may be as an ointment or cream and rectal administration may be as a suppository. For parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) the composition may take the form of, for example, a sterile solution, suspension or emulsion.

The dose of a compound of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the compound chosen, the route of administration, the form and severity of the condition and whether the compound is to be administered alone or in combination with another drug. Thus the precise dose will be determined by the administering physician but in general daily dosages may be in the range 0.001 to 100mg/kg preferably 0.01 to 50mg/kg.

BIOLOGICAL ACTIVITY

The following test was used to demonstrate the activity of compounds according to the invention:

5

Activity against tumour vasculature measured by fluorescent dye.

The following experiment further demonstrates the ability of the compounds to damage tumour vasculature.

10 Tumour functional vascular volume in CaNT tumour-bearing mice was measured using the fluorescent dye Hoechst 33342 according to the method of Smith *et al* (Brit J Cancer 57, 247-253, 1988). The fluorescent dye was dissolved in saline at 6.25 mg/ml and injected intravenously at 10 mg/kg 24 hours after drug treatment. One minute later, animals were killed and tumours excised and frozen; 10 µm sections were cut at 3 different levels and observed under UV illumination using an Olympus microscope
15 equipped with epifluorescence. Blood vessels were identified by their fluorescent outlines and vascular volume was quantified using a point scoring system based on that described by Chalkley, (J Natl Cancer Inst, 4, 47-53, 1943). All estimates were based on counting a minimum of 100 fields from sections cut at the 3 different levels.

20 Induction of necrosis

Mice bearing either CaNT or SaS tumours were treated with the test compound and tumours excised after 24h, fixed in formalin, embedded in paraffin, sectioned and stained with haematoxylin and eosin. Sections were scored based on area of necrosis
25 as follows:

% necrosis	score		% necrosis	score
0-10	1		51-60	6
11-20	2		61-70	7
21-30	3		71-80	8
31-40	4		81-90	9
41-50	5		91-100	10

Control tumours had mean scores of 2.0 (CaNT) and 1.0 (SaS). Mean values from at least three different tumours were obtained for each test compound.

Table: Reduction in Vascular Volume and Induction of Necrosis in the Carcinoma NT

- 5 Tumour 24h Post Dose: Comparison with Combretastatin A4 phosphate (CA4P).

Compound	Dose	Vascular volume % reduction	Necrosis score
CA4P	50mg/kg i.v.	88	5.7
CA4P	50mg/kg i.p.	91	6.0
Cmpd. of Example 1	50mg/kg i.v.	98	10.0
Cmpd. of Example 2	50mg/kg i.p.	95	8.0

- 10 The following non-limiting Examples illustrate the invention:

EXAMPLE 1

(Z)-1-(4-Methoxy-3-N^G-nitroarginyloxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene

- 15 Trifluoroacetic acid (0.2ml) was added to a solution of (Z)-1-(3-(N- α -t-butoxycarbonyl-N- ω -nitroarginyloxy)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (82mg) in dichloromethane (3ml) at 0°C and the mixture allowed to come to room temperature and stir 16h. The mixture was concentrated under reduced pressure, ethanol (5ml) was added, the mixture was reconcentrated
- 20 under reduced pressure and the procedure repeated three times. Trituration with diethyl ether afforded the title compound (69mg) as an off-white powder m.p. 157-159°C.

The (Z)-1-(3-(N- α -t-butoxycarbonyl-N- ω -nitroarginyloxy)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene used in the above procedure was prepared as follows: A solution of (Z)-1-(3-hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (65mg, 0.21mmol), N α -t-BOC- ω -nitro-L-arginine (134mg, 0.42mmol), 1-(3-
5 dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (110mg, 0.54mmol) and 4-dimethylaminopyridine (5mg) in dichloromethane (2.1ml) was stirred at room temperature for 72h. The reaction mixture was partitioned between dichloromethane and water and the aqueous phase extracted with two portions of dichloromethane. The combined organic extracts were washed successively with two portions of water
10 and one of brine, dried (MgSO₄) and concentrated under reduced pressure. The residue was chromatographed on silica gel eluting with 33% ethyl acetate/hexane followed by 100% ethyl acetate to give (Z)-1-(3-(N- α -t-butoxycarbonyl-N- ω -nitroarginyloxy)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (82mg) as a white oil.

EXAMPLE 2

(Z)-N-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)ethenyl]phenoxy]carbonyl]N^G-nitroarginine methyl ester

A solution of (Z)-1-(3-hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (400mg, 1.27mmol) in dry pyridine (2ml) was added dropwise to a cooled (0°C) mixture of 4-nitrophenylchloroformate (282mg, 1.40mmol) and dry pyridine (1ml). After 20min the reaction mixture was warmed to room temperature and stirred for a further 6h. To this was added L-N^G-nitroarginine methyl ester hydrochloride (343mg,
25 1.27mmol, azeotroped with toluene) and the mixture heated (70°C) for 72h. After cooling to room temperature, the reaction mixture was partitioned (ethyl acetate, water), the organic layer was washed (water x3), the aqueous layer was extracted (ethyl acetate x3), the combined organic fractions were further washed (water x2, saturated NaCl(aq) x1), dried (MgSO₄), and concentrated *in vacuo*. Flash silica
30 chromatography, eluting with 50% ethyl acetate/hexane then 100% ethyl acetate,

afforded the title compound as a white foam (292mg). Elemental analysis: calculated C 54.26% H 5.78% N 12.17%, found C 53.97% H 6.07% N 11.55%.

EXAMPLE 3

5 (Z)-N-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)ethenyl]phenoxycarbonyl]N^G-nitroarginine

A mixture of (Z)-N-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)ethenyl]phenoxycarbonyl]N^G-nitroarginine methyl ester (95mg, 0.165mmol), tetrahydrofuran (10ml), water (10ml) and concentrated sulphuric acid (1ml) were heated at 60°C for 72h. After cooling to room temperature, the reaction mixture was partitioned (ethyl acetate, water), the aqueous layer was extracted (ethyl acetate x3), the combined organic fractions were further washed (water x2, saturated NaCl(aq) x1), dried (MgSO₄), and concentrated in vacuo. The title compound was
15 obtained as an opaque oil (90mg, 98%). LC-MS indicated purity >95%.

In a similar manner to Example 2 there was prepared:

EXAMPLE 4

20 (Z)-N-[2-methyl-5-[2-(3,4,5-trimethoxyphenyl)ethenyl]phenoxycarbonyl]N^G-nitroarginine methyl ester

From (Z)-1-(3-hydroxy-4-methyl)-2-(3,4,5-trimethoxyphenyl)ethene (125mg, 0.42mmol), nitrophenylchloroformate (93mg, 0.46mmol) and L-N^G-nitroarginine methyl ester hydrochloride (113mg, 0.42mmol) there was obtained the title compound (15mg) as a colourless oil. LC-MS indicated purity >95%. MS (*m/z*) 300 (M⁺), 285.

The (Z)-1-(3-hydroxy-4-methyl)-2-(3,4,5-trimethoxyphenyl)ethene used as starting material was prepared as follows:

A suspension of 3,4,5-trimethoxybenzyltriphenylphosphonium bromide (8g, 15.3mmol) in tetrahydrofuran (450ml) at -23°C was treated with n-butyllithium (10ml of a solution in hexanes, 15.3 mmol) dropwise and the mixture stirred for 1h. 4-methoxy-

30

3-*tert*-butyldimethylsilyloxybenzaldehyde (4.07g, 15.3mmol) was added and the mixture stirred a further 4h at -23°C before warming to room temperature and stirring a further 16h. The mixture was poured on to ice-water (150ml) and extracted with diethyl ether (three portions of 150ml). The combined extracts were washed with water (three portions of 150ml) and brine (150ml), dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel eluting with 5% ethyl acetate in hexane followed by 15% ethyl acetate in hexane to give a white solid (4.61g) consisting of (Z)-1-(4-methyl-3-*tert*-butyldimethylsilyloxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene. A portion of this material (3.46g, 8mmol) was dissolved in tetrahydrofuran (60ml) and treated with tetrabutylammonium fluoride (8.3 ml of a 1.0M solution in tetrahydrofuran, 8.3mmol) and stirred for 20min. Ice (20g) was added and the mixture extracted with diethyl ether (200ml). The extract was washed with water (three portions of 80ml), dried (MgSO₄) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel eluting with 40% ethyl acetate in hexane. 1-(3-hydroxy-4-methyl)-2-(3,4,5-trimethoxyphenyl)ethene (2.01g) was obtained as a white solid.

CLAIMS:

1. A vascular damaging agent which is a compound of formula IA

5

A-X-B**IA**

Wherein

10

A is a substituted *cis*-stilbene moiety

X is a linker bond, atom or group

B is a moiety derived from an inhibitor of the formation or action of nitric oxide in mammalian systems

15

and the hydrates, pharmaceutically acceptable salts and prodrugs thereof.

2. A vascular damaging agent which is a compound of formula I

20

A-X-B**I**25

Wherein

A is a substituted *cis*-stilbene moiety

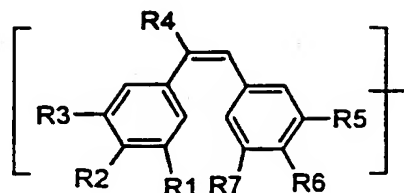
X is a linker bond, atom or group

B is a moiety derived from an inhibitor of nitric oxide synthase

30

and the hydrates, pharmaceutically acceptable salts and prodrugs thereof.

3. A vascular damaging agent according to claim 2 in which the *cis*-stilbene moiety is a group of formula II



II

Wherein

R1, R2 and R3 are each independently H, optionally substituted alkoxy, optionally substituted alkyl or halogen

- 10 R4 is hydrogen or cyano

R5, R6 and R7 are each independently H, hydroxy, optionally substituted alkoxy, optionally substituted alkyl, halogen, amino, alkylamino, dialkylamino, cyano, nitro, carboxyl, alkanoyl, alkoxycarbonyl, alkoxycarbonyloxy, alkoxycarbonylamino, aminocarbonylamino, alkylaminocarbonylamino, dialkylaminocarbonylamino, 15 alkylcarbonylamino, alkylsulphonyl, aminosulphonyl, alkylaminosulphonyl, dialkylaminosulphonyl, alkylsulphonylamino, aminosulphonylamino, alkylaminosulphonylamino, dialkylaminosulphonylamino, mercapto, alkylsulphanyl or alkylsulphinyl,

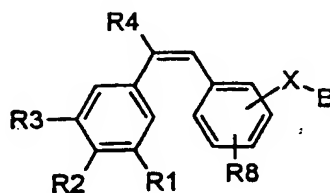
- 20 with the proviso that at least two of R1, R2 and R3 must be optionally substituted alkoxy.

4. An agent according to either of claims 2 and 3 in which the linker group X is a bond.

- 25 5. An agent according to either of claims 2 and 3 in which the linker group is selected from an optionally substituted methylene chain, or $-(CH_2)_m-Y-(CH_2)_n-$ wherein Y is selected from -O-, -S-, -S(O)-, -SO₂-, -NH-, -Nalkyl-, -CO-, -OC(O)-, -

NHC(O)-, -N(alkyl)C(O)-, -NHC(O)NH-, -NalkylC(O)NH-, -NalkylC(O)Nalkyl-, -NHSO₂-, -NalkylSO₂-, -NHSO₂NH-, -NalkylSO₂NH-, -NalkylSO₂Nalkyl- and -OC(O)O-, m is 0-3 and n is 0-3.

- 5 6. An agent according to any one of claims 2 to 5 in which the nitric oxide synthase inhibitor moiety is selected from a group derived from an amino acid inhibitor of nitric oxide synthase, a thiocitrulline derivative, an S-alkylisothiurea derivative or 2-aminopyridine derivative.
- 10 7. An agent according to claim 6 in which the group derived from an amino acid inhibitor of nitric oxide synthase is a group -C(O)CH(NH₂)-(CH₂)_p-NHC(NH)Z wherein p is 1-5 and Z is alkyl, alkylamino, dialkylamino, nitroamino, hydrazino or alkylthio, or a group -NHCH(CO₂R₁₀)-(CH₂)_p-NHC(NH)Z where p and Z are as hereinbefore described and R₁₀ is hydrogen or alkyl.
- 15 8. An agent according to claim 6 in which the thiocitrulline group is -C(O)CH(NH₂)-(CH₂)_p-NHC(S)NH₂ or a group -NHCH(CO₂R₁₀)-(CH₂)_p-NHC(S)NH₂.
- 20 9. An agent according to claim 6 in which the derivative of S-alkylisothiurea is -(CH₂)_p-SC(NH)NH₂.
10. An agent according to claim 6 in which the derivative of 2-aminopyridine is 4-methyl-2-pyridinylamino.
- 25 11. An agent according to claim 2 wherein the compound is



Wherein

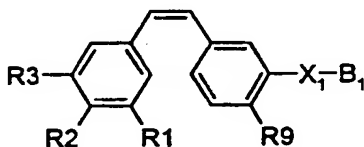
R1, R2, R3, R4, X and B are as hereinbefore described

R8 is alkyl, amino, hydroxy, alkoxy or halogen

5

12. An agent according to claim 11 wherein the compounds are of formula III wherein R1, R2, R3, R4, are as hereinbefore described, R8 is alkyl, amino, hydroxy, alkoxy or halogen, X is -O- or -NH- and B is a group -C(O)CH(NH₂)-(CH₂)_p-NHC(NH)Z wherein p is 1-5 and Z is alkyl, alkylamino, dialkylamino, nitroamino, hydrazino or alkylthio or a group -NHCH(CO₂R₁₀)-(CH₂)_p-NHC(NH)Z where p, Z and R₁₀ are as hereinbefore described.
- 10

13. An agent according to claim 1 wherein the agent is of formula



15

IV

Wherein

R1, R2 and R3 are as hereinbefore described

R9 is alkyl, alkoxy or halogen

- 20 X₁ is O or NH

B₁ is a group -C(O)CH(NH₂)-(CH₂)_p-NHC(NH)Z wherein p is 1-5 and Z is alkyl, alkylamino, dialkylamino, nitroamino, hydrazino or alkylthio.

14. An agent according to claim 2 which is selected from
- 25 (Z)-1-(4-Methoxy-3-N^G-nitroarginyloxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene
 (Z)-N-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)ethenyl]phenoxy carbonyl]N^G-nitroarginine methyl ester
 (Z)-N-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)ethenyl]phenoxy carbonyl]N^G-nitroarginine

(Z)-N-[2-methyl-5-[2-(3,4,5-trimethoxyphenyl)ethenyl]phenoxy carbonyl]N^G-nitroarginine methyl ester

15. Use of a substituted stilbene compound in preparation of a medicament for the treatment of diseases involving neovascularisation characterised in that the stilbene compound is of formula IA

A-X-B

IA

Wherein

A is a substituted *cis*-stilbene moiety

- 15 X is a linker bond, atom or group

B is a moiety derived from an inhibitor of the formation or action of nitric oxide in mammalian systems

and the hydrates, pharmaceutically acceptable salts and prodrugs thereof.

20

16. Use of a substituted stilbene compound in preparation of a medicament for the treatment of diseases involving neovascularisation characterised in that the stilbene compound is of formula I

25

A-X-B

I

Wherein

30

A is a substituted *cis*-stilbene moiety

X is a linker bond, atom or group

B is a moiety derived from an inhibitor of nitric oxide synthase

and the hydrates, pharmaceutically acceptable salts and prodrugs thereof.

17. A method for the treatment of diseases involving neovascularisation

5 characterised by the administration of a stilbene derivative of formula I



10

Wherein

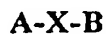
A is a substituted *cis*-stilbene moiety

X is a linker bond, atom or group

15 B is a moiety derived from an inhibitor of the formation or action of nitric oxide in mammalian systems

and the hydrates, pharmaceutically acceptable salts and prodrugs thereof.

20 18. A method for the treatment of diseases involving neovascularisation characterised by the administration of a stilbene derivative of formula I



25



Wherein

A is a substituted *cis*-stilbene moiety

30 X is a linker bond, atom or group

B is a moiety derived from an inhibitor of nitric oxide synthase

and the hydrates, pharmaceutically acceptable salts and prodrugs thereof.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 00/00503

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/195 A61P35/00 A61P17/00 A61P27/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 641 767 A (AJINOMOTO KK) 8 March 1995 (1995-03-08) abstract page 2, line 1 - line 40 examples tables 1,2 claims 1-10	1-18
X	WO 92 16486 A (ASTON MOLECULES LTD) 1 October 1992 (1992-10-01) abstract page 1, line 26 - page 3, line 25 page 3, line 27 - line 36 claims 1-18	1-18
	-/-	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

12 May 2000

Date of mailing of the international search report

25. 05. 00

Name and mailing address of the ISA

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Authorized officer

Taylor, G.M.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 00/00503

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>KOJI OHSUMI ET AL: "Novel Combretastatin Analogues Effective against Murine Solid Tumors: Design and Structure-Activity Relationships" JOURNAL OF MEDICINAL CHEMISTRY, US, AMERICAN CHEMICAL SOCIETY, WASHINGTON, vol. 41, no. 16, 30 July 1998 (1998-07-30), pages 3022-3032-3032, XP002102895 ISSN: 0022-2623 tables 1,2,4-6 Conclusion</p>	1-18
X	<p>OHSUMI K ET AL: "Syntheses and antitumor activity of cis-restricted combretastatins: 5-membered heterocyclic analogues" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, GB, OXFORD, vol. 8, no. 22, 17 November 1998 (1998-11-17), pages 3153-3158, XP004143718 ISSN: 0960-894X Introduction Compounds 4 and 5</p>	1-18
X	<p>GEORGE R PETTIT ET AL: "Antineoplastic Agents 322. Synthesis of Combretastatin A-4 Prodrugs" ANTI-CANCER DRUG DESIGN, GB, BASINGSTOKE, vol. 10, no. 4, June 1995 (1995-06), pages 299-309-309, XP002102893 ISSN: 0266-9536 Summary Introduction Compounds 1e-1j, 2 page 306 -page 308</p>	1-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/00503

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 17 and 18 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/00503

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0641767 A	08-03-1995	AT 174899 T	15-01-1999
		CA 2131683 A	09-03-1995
		CN 1105967 A,B	02-08-1995
		DE 69415445 D	04-02-1999
		DE 69415445 T	22-07-1999
		ES 2126068 T	16-03-1999
		GR 3029603 T	30-06-1999
		JP 7228558 A	29-08-1995
		SI 641767 T	30-04-1999
		US 5525632 A	11-06-1996
		US 5731353 A	24-03-1998
WO 9216486 A	01-10-1992	AU 1371992 A	21-10-1992

1. The first part of the document is a list of names and addresses of the members of the committee. The names are listed in alphabetical order, and the addresses are listed below each name. The list includes names such as Mr. John A. Smith, Mr. James B. Jones, and Mr. Robert C. Brown.

2. The second part of the document is a list of the names and addresses of the members of the committee who have been elected to the position of chairman. The names are listed in alphabetical order, and the addresses are listed below each name. The list includes names such as Mr. John A. Smith, Mr. James B. Jones, and Mr. Robert C. Brown.

3. The third part of the document is a list of the names and addresses of the members of the committee who have been elected to the position of secretary. The names are listed in alphabetical order, and the addresses are listed below each name. The list includes names such as Mr. John A. Smith, Mr. James B. Jones, and Mr. Robert C. Brown.

4. The fourth part of the document is a list of the names and addresses of the members of the committee who have been elected to the position of treasurer. The names are listed in alphabetical order, and the addresses are listed below each name. The list includes names such as Mr. John A. Smith, Mr. James B. Jones, and Mr. Robert C. Brown.

5. The fifth part of the document is a list of the names and addresses of the members of the committee who have been elected to the position of clerk. The names are listed in alphabetical order, and the addresses are listed below each name. The list includes names such as Mr. John A. Smith, Mr. James B. Jones, and Mr. Robert C. Brown.

6. The sixth part of the document is a list of the names and addresses of the members of the committee who have been elected to the position of auditor. The names are listed in alphabetical order, and the addresses are listed below each name. The list includes names such as Mr. John A. Smith, Mr. James B. Jones, and Mr. Robert C. Brown.

7. The seventh part of the document is a list of the names and addresses of the members of the committee who have been elected to the position of assessor. The names are listed in alphabetical order, and the addresses are listed below each name. The list includes names such as Mr. John A. Smith, Mr. James B. Jones, and Mr. Robert C. Brown.

8. The eighth part of the document is a list of the names and addresses of the members of the committee who have been elected to the position of collector. The names are listed in alphabetical order, and the addresses are listed below each name. The list includes names such as Mr. John A. Smith, Mr. James B. Jones, and Mr. Robert C. Brown.